

SEQUENCE LISTING



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SARA, Margit

<120> RECOMBINANT EXPRESSION OF S-LAYER PROTEINS

<130> 100564-08013

<140> 09/117,447

<141> 1998-12-02

<150> PCT/EP97/00432

<151> 1997-01-31

<150> DE/196 03 649.6

<151> 1996-02-01

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<170> PatentIn Ver. 2.1

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TECHNICAL CENTER 1800/2000

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 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: synthetic
 primer

<400> 3
 ttaatcgatt ctagatggat aggaaaaaag ctg 33

<210> 4
 <211> 37
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: synthetic
 primer

<400> 4
 atacccgggg gtacggatcc gatacagatt tgagcaa 37

<210> 5
 <211> 2766
 <212> DNA
 <213> Bacillus stearothermophilus

<220>
 <221> CDS
 <222> (1)..(2763)

<220>
 <221> sig_peptide
 <222> (1)..(93)

<220>
 <221> mat_peptide
 <222> (94)..(2763)

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 Met Ala Tyr Gln Pro Lys Ser Phe Arg Lys Phe Val Ala Thr Thr Ala
 -30 -25 -20
 aca gct gcc att gta gca tct gcg gta gct cct gta gta tct gca gca 96
 Thr Ala Ala Ile Val Ala Ser Ala Val Ala Pro Val Val Ser Ala Ala
 -15 -10 -5 -1 1
 agc ttc aca gat gtt gcg ccg caa tat aaa gat gcg atc gat ttc tta 144
 Ser Phe Thr Asp Val Ala Pro Gln Tyr Lys Asp Ala Ile Asp Phe Leu
 5 10 15
 gta tca act ggt gca aca aaa ggt aaa aca gaa aca aaa ttc ggc gtt 192
 Val Ser Thr Gly Ala Thr Lys Gly Lys Thr Glu Thr Lys Phe Gly Val
 20 25 30
 tac gat gaa atc act cgt cta gat gcg gca gtt att ctt gca aga gta 240
 Tyr Asp Glu Ile Thr Arg Leu Asp Ala Ala Val Ile Leu Ala Arg Val
 35 40 45
 tta aaa cta gac gtt gac aac gca aaa gac gca ggc ttc aca gat gtg 288
 Leu Lys Leu Asp Val Asp Asn Ala Lys Asp Ala Gly Phe Thr Asp Val
 50 55 60 65
 cca aaa gac cgt gca aaa tac gtc aac gcg ctt gta gaa gct ggc gta 336
 Pro Lys Asp Arg Ala Lys Tyr Val Asn Ala Leu Val Glu Ala Gly Val
 70 75 80
 tta aac ggt aaa gca cct ggc aaa ttt ggt gca tac gac cca tta act 384
 Leu Asn Gly Lys Ala Pro Gly Lys Phe Gly Ala Tyr Asp Pro Leu Thr
 85 90 95
 cgc gtt gaa atg gca aaa atc atc gcg aac cgt tac aaa tta aaa gct 432
 Arg Val Glu Met Ala Lys Ile Ile Ala Asn Arg Tyr Lys Leu Lys Ala

100	105	110	
gac gat gta aaa ctt cca ttc act gat gta aac gat aca tgg gca cca			480
Asp Asp Val Lys Leu Pro Phe Thr Asp Val Asn Asp Thr Trp Ala Pro			
115	120	125	
tac gta aaa gcg ctt tat aaa tac gaa gta acc aaa agg tta aaa cac			528
Tyr Val Lys Ala Leu Tyr Lys Tyr Glu Val Thr Lys Arg Leu Lys His			
130	135	140	145
caa caa gct tcg gtg cat acc aaa aac atc act ctg cgt gac ttt gcg			576
Gln Gln Ala Ser Val His Thr Lys Asn Ile Thr Leu Arg Asp Phe Ala			
150	155	160	
caa ttt gta tat aga gcg gtg aat att aat gca gtg cca gaa ata gtt			624
Gln Phe Val Tyr Arg Ala Val Asn Ile Asn Ala Val Pro Glu Ile Val			
165	170	175	
gaa gta act gcg gtt aat tcg act aca gtg aaa gta aca ttc aat acg			672
Glu Val Thr Ala Val Asn Ser Thr Thr Val Lys Val Thr Phe Asn Thr			
180	185	190	
caa att gct gat gtt gat ttc aca aat ttt gct atc gat aac ggt tta			720
Gln Ile Ala Asp Val Asp Phe Thr Asn Phe Ala Ile Asp Asn Gly Leu			
195	200	205	
act gtt act aaa gca act ctt tct cgt gat aaa aaa tcc gta gag gtt			768
Thr Val Thr Lys Ala Thr Leu Ser Arg Asp Lys Lys Ser Val Glu Val			
210	215	220	225
gtg gta aat aaa ccg ttt act cgt aat cag gaa tat aca att aca gcg			816
Val Val Asn Lys Pro Phe Thr Arg Asn Gln Glu Tyr Thr Ile Thr Ala			
230	235	240	
aca ggc att aaa aat tta aaa ggc gag acc gct aag gaa tta act ggt			864
Thr Gly Ile Lys Asn Leu Lys Gly Glu Thr Ala Lys Glu Leu Thr Gly			
245	250	255	
aag ttt gtt tgg tct gtt caa gat gcg gta act gtt gca cta aat aat			912
Lys Phe Val Trp Ser Val Gln Asp Ala Val Thr Val Ala Leu Asn Asn			
260	265	270	
agt tcg ctt aaa gtt gga gag gaa tct ggt tta act gta aaa gat cag			960
Ser Ser Leu Lys Val Gly Glu Glu Ser Gly Leu Thr Val Lys Asp Gln			
275	280	285	
gat ggc aaa gat gtt gta ggt gct aaa gta gaa ctt act tct tct aat			1008
Asp Gly Lys Asp Val Val Gly Ala Lys Val Glu Leu Thr Ser Ser Asn			

290	295	300	305	
act aat att gtt gta gtt tca agt ggc gaa gta tca gta tct gct gct				1056
Thr Asn Ile Val Val Val Ser Ser Gly Glu Val Ser Val Ser Ala Ala				
310	315	320		
aaa gtt aca gct gta aaa ccg gga aca gct gat gtt act gca aaa gtt				1104
Lys Val Thr Ala Val Lys Pro Gly Thr Ala Asp Val Thr Ala Lys Val				
325	330	335		
aca tta cca gat ggt gtt gta cta aca aat aca ttt aaa gtg aca gtt				1152
Thr Leu Pro Asp Gly Val Val Leu Thr Asn Thr Phe Lys Val Thr Val				
340	345	350		
aca gaa gtg cct gtt caa gtc caa aat caa gga ttt act tta gtt gat				1200
Thr Glu Val Pro Val Gln Val Gln Asn Gln Gly Phe Thr Leu Val Asp				
355	360	365		
aat ctt tct aat gct cca cag aat aca gtt gca ttt aac aaa gct gag				1248
Asn Leu Ser Asn Ala Pro Gln Asn Thr Val Ala Phe Asn Lys Ala Glu				
370	375	380	385	
aaa gta act tca atg ttt gct gga gaa act aaa aca gtt gca atg tat				1296
Lys Val Thr Ser Met Phe Ala Gly Glu Thr Lys Thr Val Ala Met Tyr				
390	395	400		
gat act aaa aac ggt gat cct gaa act aaa cct gtt gat ttc aaa gat				1344
Asp Thr Lys Asn Gly Asp Pro Glu Thr Lys Pro Val Asp Phe Lys Asp				
405	410	415		
gca act gta cgt tca tta aat cca att att gca aca gct gct att aat				1392
Ala Thr Val Arg Ser Leu Asn Pro Ile Ile Ala Thr Ala Ala Ile Asn				
420	425	430		
ggt agt gag ctc ctt gtc aca gct aat gct ggc caa tct gga aaa gct				1440
Gly Ser Glu Leu Leu Val Thr Ala Asn Ala Gly Gln Ser Gly Lys Ala				
435	440	445		
tca ttt gaa gta aca tta aaa gat aat aca aaa aga aca ttt aca gtt				1488
Ser Phe Glu Val Thr Leu Lys Asp Asn Thr Lys Arg Thr Phe Thr Val				
450	455	460	465	
gat gta aaa aaa gac cct gta tta caa gat ata aaa gta gat gca act				1536
Asp Val Lys Lys Asp Pro Val Leu Gln Asp Ile Lys Val Asp Ala Thr				
470	475	480		
tct gtt aaa ctt tcc gat gaa gct gtt ggc ggc ggg gaa gtt gaa gga				1584
Ser Val Lys Leu Ser Asp Glu Ala Val Gly Gly Gly Glu Val Glu Gly				

485	490	495	
ggt aac caa aaa acg att aaa gta agt gca gtt gac caa tac ggt aaa			1632
Val Asn Gln Lys Thr Ile Lys Val Ser Ala Val Asp Gln Tyr Gly Lys			
500	505	510	
gaa att aaa ttt ggt aca aaa ggt aaa gtt act gtt aca act aat aca			1680
Glu Ile Lys Phe Gly Thr Lys Gly Lys Val Thr Val Thr Thr Asn Thr			
515	520	525	
gaa gga cta gtt att aaa aat gta aat agc gat aat aca att gac ttt			1728
Glu Gly Leu Val Ile Lys Asn Val Asn Ser Asp Asn Thr Ile Asp Phe			
530	535	540	545
gat agc ggc aat agt gca act gac caa ttt gtt gtc gtt gca aca aaa			1776
Asp Ser Gly Asn Ser Ala Thr Asp Gln Phe Val Val Val Ala Thr Lys			
550	555	560	
gac aaa att gtc aat ggt aaa gta gaa gtt aaa tat ttc aaa aat gct			1824
Asp Lys Ile Val Asn Gly Lys Val Glu Val Lys Tyr Phe Lys Asn Ala			
565	570	575	
agt gac aca aca cca act tca act aaa aca att act gtt aat gta gta			1872
Ser Asp Thr Thr Pro Thr Ser Thr Lys Thr Ile Thr Val Asn Val Val			
580	585	590	
aat gta aaa gct gac gct aca cca gta gga tta gat att gta gca cct			1920
Asn Val Lys Ala Asp Ala Thr Pro Val Gly Leu Asp Ile Val Ala Pro			
595	600	605	
tct aaa att gat gta aat gct cca aac act gct tct act gca gat gtt			1968
Ser Lys Ile Asp Val Asn Ala Pro Asn Thr Ala Ser Thr Ala Asp Val			
610	615	620	625
gat ttt ata aat ttc gaa agt gtt gag att tac aca ctc gat tca aat			2016
Asp Phe Ile Asn Phe Glu Ser Val Glu Ile Tyr Thr Leu Asp Ser Asn			
630	635	640	
ggt aga cgt caa aaa aaa gtt act cca act gca act aca ctt gta ggt			2064
Gly Arg Arg Gln Lys Lys Val Thr Pro Thr Ala Thr Thr Leu Val Gly			
645	650	655	
aca aaa aaa aaa aaa aaa gtt aat ggg aat gta tta caa ttc aag ggg			2112
Thr Lys Lys Lys Lys Lys Val Asn Gly Asn Val Leu Gln Phe Lys Gly			
660	665	670	
aac gaa gaa tta acg cta tca act tct tct agt aca gga aac gta gat			2160
Asn Glu Glu Leu Thr Leu Ser Thr Ser Ser Ser Thr Gly Asn Val Asp			

675	680	685	
gga aca gca gaa gga atg aca aaa cgt att cca ggg aaa tat atc aac			2208
Gly Thr Ala Glu Gly Met Thr Lys Arg Ile Pro Gly Lys Tyr Ile Asn			
690	695	700	705
tct gca agt gta cct gcc agt gca aca gta gca aca agt cct gtt act			2256
Ser Ala Ser Val Pro Ala Ser Ala Thr Val Ala Thr Ser Pro Val Thr			
	710	715	720
gta aag ctt aat tca agt gat aat gat tta aca ttt gaa gaa tta ata			2304
Val Lys Leu Asn Ser Ser Asp Asn Asp Leu Thr Phe Glu Glu Leu Ile			
	725	730	735
ttc ggt gta att gac cct aca caa tta gtc aaa gat gaa gac atc aac			2352
Phe Gly Val Ile Asp Pro Thr Gln Leu Val Lys Asp Glu Asp Ile Asn			
	740	745	750
gaa ttt att gca gtt tca aaa gcg gct aaa aat gat gga tat ttg tat			2400
Glu Phe Ile Ala Val Ser Lys Ala Ala Lys Asn Asp Gly Tyr Leu Tyr			
	755	760	765
aat aaa ccg ctt gta acg gtt aaa gat gca tca gga aaa gtt att cca			2448
Asn Lys Pro Leu Val Thr Val Lys Asp Ala Ser Gly Lys Val Ile Pro			
770	775	780	785
aca ggt gca aat gtt tac ggt cta aat cat gat gca act aac gga aac			2496
Thr Gly Ala Asn Val Tyr Gly Leu Asn His Asp Ala Thr Asn Gly Asn			
	790	795	800
att tgg ttt gat gag gaa caa gct ggc tta gct aaa aaa ttt agt gat			2544
Ile Trp Phe Asp Glu Glu Gln Ala Gly Leu Ala Lys Lys Phe Ser Asp			
	805	810	815
gta cat ttt gat gtt gat ttt tca tta act aac gtt gta aaa act ggt			2592
Val His Phe Asp Val Asp Phe Ser Leu Thr Asn Val Val Lys Thr Gly			
	820	825	830
agc ggt aca gtt tct tca tcg cca tca tta tct gac gca att caa ctt			2640
Ser Gly Thr Val Ser Ser Ser Pro Ser Leu Ser Asp Ala Ile Gln Leu			
835	840	845	
act aat tca ggc gat gca gta tcg ttt aca tta gtt atc aaa tca att			2688
Thr Asn Ser Gly Asp Ala Val Ser Phe Thr Leu Val Ile Lys Ser Ile			
850	855	860	865
tat gtt aaa ggc gca gat aaa gat gat aat aac tta ctt gca gcc cct			2736
Tyr Val Lys Gly Ala Asp Lys Asp Asp Asn Asn Leu Leu Ala Ala Pro			

870 875 880
 gtt tct gtc aat gtg act gtg aca aaa taa 2766
 Val Ser Val Asn Val Thr Val Thr Lys
 885 890

 <210> 6
 <211> 921
 <212> PRT
 <213> *Bacillus stearothermophilus*

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 -15 -10 -5 -1 1
 Ser Phe Thr Asp Val Ala Pro Gln Tyr Lys Asp Ala Ile Asp Phe Leu
 5 10 15
 Val Ser Thr Gly Ala Thr Lys Gly Lys Thr Glu Thr Lys Phe Gly Val
 20 25 30
 Tyr Asp Glu Ile Thr Arg Leu Asp Ala Ala Val Ile Leu Ala Arg Val
 35 40 45
 Leu Lys Leu Asp Val Asp Asn Ala Lys Asp Ala Gly Phe Thr Asp Val
 50 55 60 65
 Pro Lys Asp Arg Ala Lys Tyr Val Asn Ala Leu Val Glu Ala Gly Val
 70 75 80
 Leu Asn Gly Lys Ala Pro Gly Lys Phe Gly Ala Tyr Asp Pro Leu Thr
 85 90 95
 Arg Val Glu Met Ala Lys Ile Ile Ala Asn Arg Tyr Lys Leu Lys Ala
 100 105 110
 Asp Asp Val Lys Leu Pro Phe Thr Asp Val Asn Asp Thr Trp Ala Pro
 115 120 125
 Tyr Val Lys Ala Leu Tyr Lys Tyr Glu Val Thr Lys Arg Leu Lys His
 130 135 140 145
 Gln Gln Ala Ser Val His Thr Lys Asn Ile Thr Leu Arg Asp Phe Ala
 150 155 160

Gln Phe Val Tyr Arg Ala Val Asn Ile Asn Ala Val Pro Glu Ile Val
 165 170 175
 Glu Val Thr Ala Val Asn Ser Thr Thr Val Lys Val Thr Phe Asn Thr
 180 185 190
 Gln Ile Ala Asp Val Asp Phe Thr Asn Phe Ala Ile Asp Asn Gly Leu
 195 200 205
 Thr Val Thr Lys Ala Thr Leu Ser Arg Asp Lys Lys Ser Val Glu Val
 210 215 220 225
 Val Val Asn Lys Pro Phe Thr Arg Asn Gln Glu Tyr Thr Ile Thr Ala
 230 235 240
 Thr Gly Ile Lys Asn Leu Lys Gly Glu Thr Ala Lys Glu Leu Thr Gly
 245 250 255
 Lys Phe Val Trp Ser Val Gln Asp Ala Val Thr Val Ala Leu Asn Asn
 260 265 270
 Ser Ser Leu Lys Val Gly Glu Glu Ser Gly Leu Thr Val Lys Asp Gln
 275 280 285
 Asp Gly Lys Asp Val Val Gly Ala Lys Val Glu Leu Thr Ser Ser Asn
 290 295 300 305
 Thr Asn Ile Val Val Val Ser Ser Gly Glu Val Ser Val Ser Ala Ala
 310 315 320
 Lys Val Thr Ala Val Lys Pro Gly Thr Ala Asp Val Thr Ala Lys Val
 325 330 335
 Thr Leu Pro Asp Gly Val Val Leu Thr Asn Thr Phe Lys Val Thr Val
 340 345 350
 Thr Glu Val Pro Val Gln Val Gln Asn Gln Gly Phe Thr Leu Val Asp
 355 360 365
 Asn Leu Ser Asn Ala Pro Gln Asn Thr Val Ala Phe Asn Lys Ala Glu
 370 375 380 385
 Lys Val Thr Ser Met Phe Ala Gly Glu Thr Lys Thr Val Ala Met Tyr
 390 395 400
 Asp Thr Lys Asn Gly Asp Pro Glu Thr Lys Pro Val Asp Phe Lys Asp
 405 410 415

Ala Thr Val Arg Ser Leu Asn Pro Ile Ile Ala Thr Ala Ala Ile Asn
 420 425 430

Gly Ser Glu Leu Leu Val Thr Ala Asn Ala Gly Gln Ser Gly Lys Ala
 435 440 445

Ser Phe Glu Val Thr Leu Lys Asp Asn Thr Lys Arg Thr Phe Thr Val
 450 455 460 465

Asp Val Lys Lys Asp Pro Val Leu Gln Asp Ile Lys Val Asp Ala Thr
 470 475 480

Ser Val Lys Leu Ser Asp Glu Ala Val Gly Gly Gly Glu Val Glu Gly
 485 490 495

Val Asn Gln Lys Thr Ile Lys Val Ser Ala Val Asp Gln Tyr Gly Lys
 500 505 510

Glu Ile Lys Phe Gly Thr Lys Gly Lys Val Thr Val Thr Thr Asn Thr
 515 520 525

Glu Gly Leu Val Ile Lys Asn Val Asn Ser Asp Asn Thr Ile Asp Phe
 530 535 540 545

Asp Ser Gly Asn Ser Ala Thr Asp Gln Phe Val Val Val Ala Thr Lys
 550 555 560

Asp Lys Ile Val Asn Gly Lys Val Glu Val Lys Tyr Phe Lys Asn Ala
 565 570 575

Ser Asp Thr Thr Pro Thr Ser Thr Lys Thr Ile Thr Val Asn Val Val
 580 585 590

Asn Val Lys Ala Asp Ala Thr Pro Val Gly Leu Asp Ile Val Ala Pro
 595 600 605

Ser Lys Ile Asp Val Asn Ala Pro Asn Thr Ala Ser Thr Ala Asp Val
 610 615 620 625

Asp Phe Ile Asn Phe Glu Ser Val Glu Ile Tyr Thr Leu Asp Ser Asn
 630 635 640

Gly Arg Arg Gln Lys Lys Val Thr Pro Thr Ala Thr Thr Leu Val Gly
 645 650 655

Thr Lys Lys Lys Lys Lys Val Asn Gly Asn Val Leu Gln Phe Lys Gly
 660 665 670

Asn Glu Glu Leu Thr Leu Ser Thr Ser Ser Ser Thr Gly Asn Val Asp
 675 680 685

Gly Thr Ala Glu Gly Met Thr Lys Arg Ile Pro Gly Lys Tyr Ile Asn
 690 695 700 705

Ser Ala Ser Val Pro Ala Ser Ala Thr Val Ala Thr Ser Pro Val Thr
 710 715 720

Val Lys Leu Asn Ser Ser Asp Asn Asp Leu Thr Phe Glu Glu Leu Ile
 725 730 735

Phe Gly Val Ile Asp Pro Thr Gln Leu Val Lys Asp Glu Asp Ile Asn
 740 745 750

Glu Phe Ile Ala Val Ser Lys Ala Ala Lys Asn Asp Gly Tyr Leu Tyr
 755 760 765

Asn Lys Pro Leu Val Thr Val Lys Asp Ala Ser Gly Lys Val Ile Pro
 770 775 780 785

Thr Gly Ala Asn Val Tyr Gly Leu Asn His Asp Ala Thr Asn Gly Asn
 790 795 800

Ile Trp Phe Asp Glu Glu Gln Ala Gly Leu Ala Lys Lys Phe Ser Asp
 805 810 815

Val His Phe Asp Val Asp Phe Ser Leu Thr Asn Val Val Lys Thr Gly
 820 825 830

Ser Gly Thr Val Ser Ser Ser Pro Ser Leu Ser Asp Ala Ile Gln Leu
 835 840 845

Thr Asn Ser Gly Asp Ala Val Ser Phe Thr Leu Val Ile Lys Ser Ile
 850 855 860 865

Tyr Val Lys Gly Ala Asp Lys Asp Asp Asn Asn Leu Leu Ala Ala Pro
 870 875 880

Val Ser Val Asn Val Thr Val Thr Lys
 885 890

<210> 7

<211> 498

<212> DNA

<213> Unknown Organism

<220>

<223> Description of Unknown Organism: streptavidin gene

<400> 7

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ggcacctggt ataaccaact ggggtcgact ttcattgtga ccgctggtgc ggacggagct 120
ctgactggca cctacgaatc tgcggttggt aacgcagaat cccgctacgt actgactggc 180
cgttatgact ctgcacctgc caccgatggc tctggtaccg ctctgggctg gactgtggct 240
tggaanaaca actatcgtaa tgcgcacagc gccactacgt ggtctggcca atacgttggc 300
ggtgctgagg ctcttatcaa cactcagtgg ctgttaacat ccggcactac cgaagcgaat 360
gcatggaaat cgacactagt aggtcatgac acctttacca aagttaagcc ttctgctgct 420
agcattgatg ctgccaagaa agcaggcgta aacaacggta accctctaga cgctgttcag 480
caataataag gatccggg                                     498
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<210> 8

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic primer

<400> 8

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<210> 9

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic primer

<400> 9

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agggaatat atcaactctg caagtg                                     26
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<210> 10

<211> 49

<212> DNA

<213> Bacillus stearothermophilus

<400> 10

gaattcatcg atgtcgacca aggaggtcta gatggatccg gccaaagctt

49

B^{*}
cont